COMPARATIVE ANALYSIS OF INOCULUM BIOMASS FOR BIOGAS POTENTIAL IN THE ANAEROBIC DIGESTION

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Lucrarea prezintă o analiză comparativă privind încărcarea microbiană pentru două dintre materialele cel mai frecvent utilizate ca inocul în instalaţiile de biogaz: nămolul de epurare şi dejecţiile de vite. A fost determinat conţinutul de acidogene şi metanogene utilizând tehnica statistica MPN, în funcţie de numărul de tuburi pozitive care au demonstrat un proces fermentativ, evidenţiat prin virarea culorii mediului de cultură sau prin acumularea de gaz în tuburile Durham. Rezultatele vor fi utilizate pentru cercetări suplimentare privind selectarea tipului adecvat de biomasă de inocul care poate conduce la obţinerea de biogaz de calitate superioară.

This paper presents a comparative analysis regarding the microbial loading of two materials most frequently used as inoculums in biogas units: sewage sludge and cattle dung. The acidogens and methanogens content has been investigated using MPN statistical technique function of the number of positive tubes which demonstrated a fermentative processes and which was pointed out either by colour change of the broth, or by gas accumulation in Durham tubes. The results will be used for further research concerning selection of the adequate inoculums biomass that can lead to obtaining of high quality biogas.

Keywords: biogas, anaerobic digestion, acidogens, methanogens

1. Introduction

During last decades, the anaerobic digestion has been considered as an attractive biotechnological process for degrading a variety of polluting organic wastes. However, the benefits of this technology are not restricted to only removal of contaminants, but energy savings can be obtained as well by computing the biogas produced (fuel methane) in a global process analysis [1]. The estimation of net energy yields is rather complex in view of the factors affecting biogas yields.

In general, the treatment processes have to be stable, low in cost and to provide an effluent quality to comply with the increasingly stringent discharge standards. These needs can be met by a better understanding of the factors

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affecting the biochemical processes and optimizing the operation of the treatment facilities.

Since anaerobic digestion is a biochemical process driven by consortia of various microorganisms which are involved in the transformation of complex high-molecular-weight organic compounds to methane, any stress or disturbance on the system may lead to a change in species types and their relative population levels, which is ultimately reflected in the reactor performance.

Therefore, one of the most important factors affecting anaerobic digestion of organic waste is the organic material added as inoculums in the fermentative organic substrate. High microbial loading inoculums material needs to be added in the mixture of biomass when start-up the anaerobic digester.

Acidogens and methanogens are the dominant species implicated in the final steps of the anaerobic digestion. It is well known that there are antibiosis interactions between these two species. Acidogens are fast growers and very little sensitive to the environment conditions variations, while methanogens are intrinsically slow growers, and are inhibited at acidic pH values. Thus, to achieve overall high rates, both the production and consumption of acids should be in balance, since an imbalance causes a slowing overall degradation of waste.

The research concerning the effect of inoculums to biogas production was conducted by several researchers with the results i.e. inoculums are substantially relevant in process kinetics of biogas production [3]; amount of methane produced seemed proportional to the initial inoculums [4]; the higher percentage of inoculums gave the higher production of biogas [5]; and the food to inoculums ration significantly affected the biogas production rate [2], [6].

Inoculums biomass is very important not only for start-up of anaerobic digesters, but also during long lasting biochemical processes, since it has been noticed that anaerobic digestion of organic matter such as municipal solid waste was not completely stopped even after 360 days of observation [7].

This study aims to investigate the methanogens vs. acidogens content of two types of biomass, successfully used as inoculums for start-up of anaerobic digesters: sewage sludge provided by municipal wastewater treatment plant and cattle dung.

The results of the comparative study regarding the microbial balance for these materials and some conclusion on the proper inoculums material which is most recommended to be used will be discussed in this paper.

2. Materials and methods

Well defined volumes of sewage sludge and cattle dung were collected in aseptic conditions and were refrigerated at 4^0C until the samples were sent to the laboratory for chemical and microbiological analysis. Six decimal dilutions were

prepared for each of these inoculums materials. For sample dilution it has been used buffered saline solution having the following chemical composition: 8.00 g of NaCl, 0.20 g of KCl, 1.44 g of Na_2HPO_4, 0.24 g of KH_2PO_4 and distilled H_2O up to 1 litter.

The initial suspension and the following six dilutions have been inoculated in three serial tubes according to the national specifications [9].

For cultivation and isolation of acidogens it has been used Hugs and Leifson's broth which contained 0.5% NaCl, 0.2% peptone, 0.03%, K_2HPO4, 0.3% agar, 0.2% bromothymol blue, 1% sugar. For methanogens growth and analysis it has been used a specific culture medium which consisted of a basal medium enriched with 1% (final concentration) mixture of sodium acetate, sodium formiate and methanol. Any increase in the oxygen content was indicated by the redox indicator sodium resazurin which changed its colour to red when oxygen was present.

3. Experimental results

The tubes of medium were heated in boiling water for 10 minutes to drive off oxygen and then were cooled and inoculated with the specific biomass, by introducing 1 ml sample of each dilution into the broth, close to the bottom of the tube. In order to create the anaerobic conditions, the surface of the medium was sealed with a layer of sterile liquid paraffin oil.

The serial tubes were aerobically incubated at 37^0C for 72 hours using a controlled temperature incubator, as seen in Figure 1. The samples were inspected daily for colour change in case of acidogens presence or for gas collection inside the Durham tubes in case of methanogens.

Fig. 1. Incubation of samples in temperature controlled environment

After incubation, the pattern of positive and negative tubes was noted for acidogens, respectively methanogens, and a standardized MPN table was

consulted to determine the most probable number of organisms, causing the positive results, per unit volume of the original sample.

The activity of acidogens and methanogens was estimated function of the positive tubes where fermentative processes could be noticed. In case of the acidogens analysis, the fermentative reactions could be made evident by the change of medium colour from dark blue-green to yellow and gas presence, as seen in Figure 2(a). The negative tubes kept their initial aspect, showing no any colour modification.

The positive tubes which indicated the presence of methanogens in the two samples were pointed out function of the visible gas bubbles accumulated in Durham tubes and above the paraffin oil layer, as well as the gelatinous sediment settled down to the bottom of the tube, as seen in Figure 2(b).

The negative tubes which did not show any methanogens content are those marked with a red layer due to the resazurin indicator which changed its colour in the presence of oxygen.

(a) (b)

Fig. 2. Positive and negative tubes for acidogens (a) and methanogens (b) analysis

Table I shows comparatively the most probable numbers of acidogens, respectively methanogens per 1ml of tested materials, corresponding to the number of positive tubes in the fermentative tests after 24, 48 and 72 h of incubation. The experimental results are plotted in Figure 3 and Figure 4.

From the experimental data it can be noticed that methanogens loading of sewage sludge is higher than of cattle dung sample. Therefore, municipal sewage sludge is suitable for being used as inoculums in anaerobic digesters, due to its high level of methanogens content which can dominate over the other antagonistic microbial species contained in the organic substrate.

Table 1

Acidogens and methanogens content of sewage sludge and cattle dung samples

Sample	**Acidogens** [cells/ml]			**Methanogens** [cells/ml]		
	24 h	48 h	72 h	24 h	48 h	72 h
Sewage sludge	4.3×10^3	2.1×10^4	1.5×10^5	1.5×10^2	7.5×10^3	6.4×10^4
Cattle dung	2.1×10^5	4.3×10^6	9.9×10^6	7.5×10	1.5×10^2	3.8×10^2

On the other hand, cattle dung contains also methanogens and can be used as organic material to start-up the anaerobic digesters, but the biogas yields will be lower in this case comparing to the situation of selecting sewage sludge as inoculums.

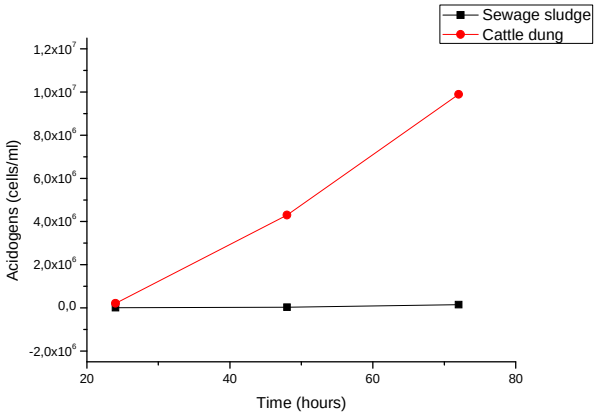

Fig. 3. Acidogens loading of sewage sludge and cattle dung

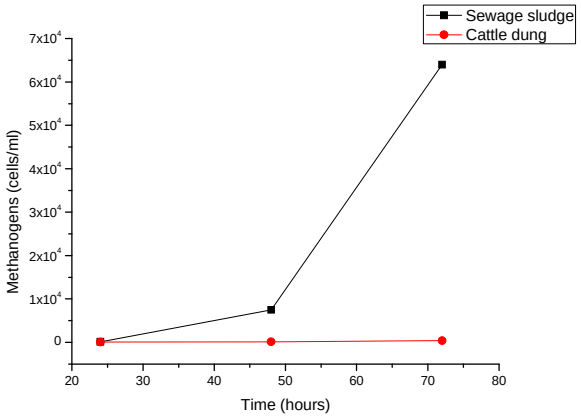

Fig. 4. Methanogens loading of sewage sludge and cattle dung

4. Conclusion

In anaerobic digestion, microorganisms digest the organic material producing biogas which can be collected and used as a valuable fuel. The yield of biogas depends on the composition of the waste feedstock and the environment conditions within the reactor.

Experimental studies revealed that the inoculums material added into organic substrate plays an essential role for start-up of the biochemical processes

from anaerobic digesters. Different types of inoculums act more or less towards enhancing the decomposition of biomass to biogas generation function of their microbial content. In order to reach high biogas yield in biogas systems, type and quality of inoculums must be known and considered prior to start the operation of any anaerobic digester. Some of the frequently used inoculums materials are sewage sludge and cattle dung due to their methanogens content and high biogas potential.

A comparative assessment of sewage sludge provided by municipal wastewater treatment plant versus cattle dung regarding the acidogens and methanogens balance has been carried out under this research work. The experimental results showed a better methanogens loading of the sewage sludge comparing to the cattle dung sample. In contrast, cattle dung analysis indicated a high level of acidogens which are inhibitors species for methanogenesis. These data pointed out the fact that sewage sludge is more suitable to be used as inoculums in anaerobic digesters than cattle slurry. Before deciding to use one or another of these two inoculums materials, price and availability criteria need to be considered.

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